# STUDIES ON THE ACROSOME.
# III. EFFECT OF CALCIUM DEFICIENCY[1]

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The idea that calcium is necessary for fertilization was clearly formulated by Jacques Loeb (1914, 1915), who suggested, as a possible explanation, that it might be an ingredient of a sort of cement which holds the sperm to the egg surface in the first stage of sperm attachment.

A very different and more complicated role in the fertilization reaction has been attributed to the calcium ion by Heilbrunn, as a corollary of his general theory of stimulation (for summary, see Heilbrunn, 1937, chaps. IX and XXXVII). In particular the work of his student, Moser (1939), showed that the breakdown of the cortical granules of sea urchin eggs, which is the precursor of membrane elevation and subsequent developmental processes in normal fertilization and artificial parthenogenesis, does not occur when the eggs are activated in oxalated or citrated media. Yamamoto (1954), pushing this analysis a step further, has found that in teleost eggs calcium ions are necessary for the propagation of an invisible "fertilization-wave" which precedes and sets off the cortical changes. Sugiyama (1953) has shown that a similar wave of excitation occurs on fertilization in the sea urchin egg.

This demonstrated importance of calcium for the first detectable change in the egg cortex at the time of activation, together with the fact that spermatozoa are normally active and attack the unfertilized egg surface vigorously even in the absence of calcium, has led to a general assumption that the failure of fertilization in Ca-free media is due to the inability of the egg to respond to the stimulus of the spermatozoan, rather than to any reduction of spermatozoan stimulating capacity in the absence of calcium.

No doubt because of the lack of a suitable method, very little effort seems to have been made to determine the effect of calcium deficiency on spermatozoa, although Tyler mentions the fact that "the absence of (calcium) interferes with the agglutination of sea urchin sperm" (1948, p. 210). The reaction of the sea urchin sperm acrosome to egg-water (Dan, 1952), however, in which at least part of the acrosomal substance is released, accompanied by the projection of a fibrous structure, provides one clear-cut criterion for comparing the effects on the individual spermatozoan of various non-fatal experimental conditions, including calcium deficiency.

The observations presented in this paper will show that calcium deficiency prevents the acrosome reaction in response to various stimuli, while it tends to enhance the agglutinating effect of egg-water and prevents the loss of fertilizing capacity after reversal of agglutination.

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## Materials and Methods

Among the sea urchins easily available at Misaki, the spermatozoa of the three species of regular sea urchins, *Hemicentrotus* (*Strongylocentrotus*) *pulcherrimus*, *Anthocidaris* (*Heliocidaris*) *crassispina* and *Pseudocentrotus depressus*, show typical reversible agglutination when mixed with the egg-water of their respective species; the following observations were made on the gametes of these three species.

Eggs were obtained by the introduction of isotonic KCl into the body cavity. In the case of "Ca-low egg-water," the animal was rinsed with Ca-free artificial sea water and made to shed its eggs directly into this solution. The egg suspension was sometimes warmed to about 30° C. to hasten dissolution of the jelly, or the eggs were centrifuged at 2000 × gravity for 5 minutes and the empty jelly hull suspension warmed to about 50° C. for a few minutes. In every case the egg-water was filtered before use.

Control egg-water solutions were obtained either by centrifuging the jelly from an equivalent volume of eggs in sea water, or by adding sufficient isotonic $CaCl_2$ to the Ca-low egg-water to bring its calcium content to that of normal sea water.

Spermatozoa were collected "dry" as shed following KCl stimulation, or as they exuded from excised testes. Care was always taken to reduce contamination by sea water or body fluids. In most of the experiments, 1 or 2% of dry sperm were suspended in Ca-free solution or filtered sea water. Spermatozoa were always freshly suspended immediately before the addition of egg-water, unless otherwise specified.

Although many biological systems are sensitive to minute changes in calcium concentration, the sperm acrosome was found to be unexpectedly insensitive in this respect. For example, in *Pseudocentrotus*, the acrosome reaction was still suppressed in all sperm at 25% of the normal calcium concentration, only about half the acrosomes reacted at 50% calcium concentration, and response of all the acrosomes was only obtained at 67% of the calcium concentration of normal sea water. In view of this fact, no effort was made in these experiments to remove the last traces of calcium from the experimental solutions by the use of oxalate or citrate, and the term "Ca-free" has therefore been avoided in referring to egg-water solutions and sperm suspensions. However, it should be pointed out that the calcium content of these media was below the threshold required for fertilization to take place in them.

As in the first study of this series, sperm suspensions were fixed by the addition of neutralized formalin. Preparations for electron microscopy were made directly from these suspensions; washing out of the sea water salts was done after mounting on the collodion membranes. Some of the preparations were shadowed with $Cr_2O_3$; the electron microscope used was a Hitachi Standard, operating on 50 KV.

## Results

The reversible agglutination of the spermatozoa of these three sea urchin species follows the usual pattern, which has been described in detail by Jacqus Loeb (1914) for two species of *Strongylocentrotus*. While there are certain details of the agglutination reaction which seem to be peculiar to one or another of the species

under consideration, description of these will be omitted, and the major aspects of the reaction common to all the species will be reported. Unless otherwise specified, the statements will apply to all three species.

Attempts to treat the agglutination reaction quantitatively run into many difficulties because of the lack of a clear end-point in the reaction itself and the virtual impossibility of standardizing the reactants (see Goldforb, 1929a, 1929b, 1929c, for a detailed discussion of individual variation among sea urchin gametes and the effect of ageing of spermatozoa on agglutination). In order to report the phenomenon with any semblance of clarity, therefore, it has seemed necessary to disregard the borderline data and concentrate attention on the cases which consistently give positive or negative results.

*Spermatozoa suspended in Ca-free sea water*

If spermatozoa freshly suspended in Ca-free sea water are compared under the high power of the phase contrast microscope with a similar sample in ordinary sea water, a striking difference is immediately observable between the behavior of the sperm in the two suspensions. The majority of the spermatozoa in sea water are either trapped against the glass surfaces on their sides or have undergone the acrosome reaction on contact with the glass and are rotating around their tips, held by the acrosome process (Dan, 1952). In the Ca-low preparation, on the other hand, all the spermatozoa are found to be in vigorous locomotion, describing uniformly-sized circles against the glass surfaces. When these have stopped moving sufficiently to permit close observation, it can be seen that none of them has reacted to the contact stimulus.

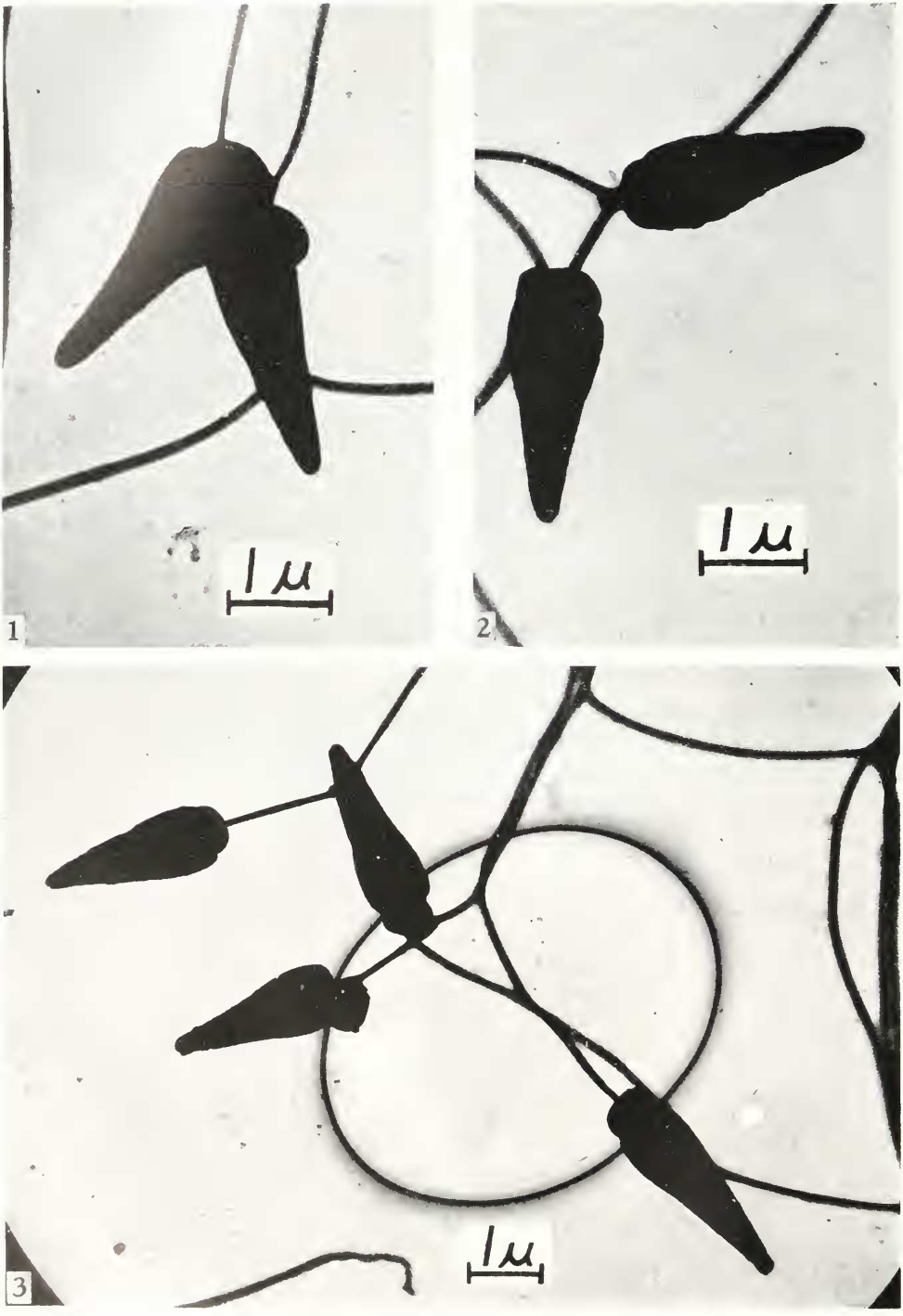
A comparison of Figures 1 and 2, electron micrographs of *Hemicentrotus* spermatozoa in sea water and Ca-free artificial sea water, respectively, shows that there is no immediate effect of lack of calcium on the sperm structure. Moreover, Figure 11 indicates that at least 10 minutes' exposure to a Ca-low medium does not produce any particular change in the appearance of the apical part of the spermatozoan (compare Fig. 8).

There is a marked tendency in Ca-low suspensions for the middle piece to be dislocated laterally from its normal position, and sometimes it moves completely around to the side of the head. This effect usually appears in most of the sperm after two or three minutes in a Ca-low medium (Fig. 11).[2] Such spermatozoa showed no diminution in either activity or fertilizing capacity (when used to inseminate eggs in normal sea water).

*Agglutination reaction in Ca-low sea water*

When Ca-low egg-water is added to a suspension of spermatozoa in Ca-free sea water, the appearance of the resulting agglutination is essentially the same as that in sea water, with the slight difference that while practically all the spermatozoa are involved in the clusters at the height of the reaction (from 5 to 30 seconds after the addition of egg-water) in sea water, a somewhat larger number of spermatozoa

[2] In this particular case only one spermatozoan shows a slight shift in the position of the middle piece; it appears much more strikingly in Figures 10 and 12, in which the effect may have been enhanced by the addition of egg-water (see Discussion).




FIGS. 1–3.

can be seen swimming freely between the clumps in Ca-low suspensions. In this medium, also, the suspended clumps are strikingly regular in shape, appearing to be perfectly spherical, in contrast to the more irregular masses formed in sea water agglutination.

One of the characteristics of reversible agglutination which is measurable to some extent is the duration of clumping. In all three of the species under consideration, it was found that, in general, a longer time was required in Ca-low than in sea water suspensions for reversal of agglutination. In *Hemicentrotus* this effect was only obtained with concentrated egg-water, such that in the sea water control a residue of small, permanently agglutinated clumps was left after the bulk of the suspension had returned to a free-swimming state. In *Anthocidaris,* Ca-low agglutination lasted 3–4 times as long as in the sea water controls, and in *Pseudocentrotus,* about twice as long.

Another quantitative comparison which can be made is that the effectiveness of a given solution of egg-water falls off much more rapidly with successive dilutions in the absence of calcium. For example, a lot of Ca-low egg-water which causes just perceptible (with low power microscopic observation of 1–2 cc. of suspension in a Syracuse watch-glass) agglutination of a Ca-low sperm suspension at a dilution of 1 : 64 may, after adjustment of its calcium content to that of normal sea water, cause an approximately equal agglutination of spermatozoa suspended in sea water at a dilution of 1 : 512.

In a sample of agglutinated sperm suspension in sea water, observed between slide and cover glass with high phase contrast magnification, there are practically no free-swimming spermatozoa, since the sperm which are not involved in the clumps are found held to one of the glass surfaces by their acrosome processes. In a sample taken after reversal of agglutination, nearly all the spermatozoa become attached to the glass, and the fact that their acrosomes have reacted is clearly observable. Electron microscopical observation of such suspensions after fixation shows all the spermatozoa with acrosome processes (Dan, 1952, Fig. 1).

In a similar sample in Ca-free sea water, however, all the sperm which are not held in the clumps are swimming freely, exactly as in Ca-low suspension before the addition of egg-water. Continuous observation of a small clump shows that while the total number of individuals in the aggregation does not noticeably increase or decrease, there is a constant coming and going of the peripheral members of the cluster. What appears to be the same behavior on a small scale is seen in certain more or less radial groupings of a few (5–10) spermatozoa at a time, at fixed points in the microscopic field (with the focus adjusted to the underside of the cover glass) where no object can be detected to form a center for such groups. The highly active spermatozoa abruptly join the group, their heads remaining more or less stationary for a few seconds or even minutes, although their tails are

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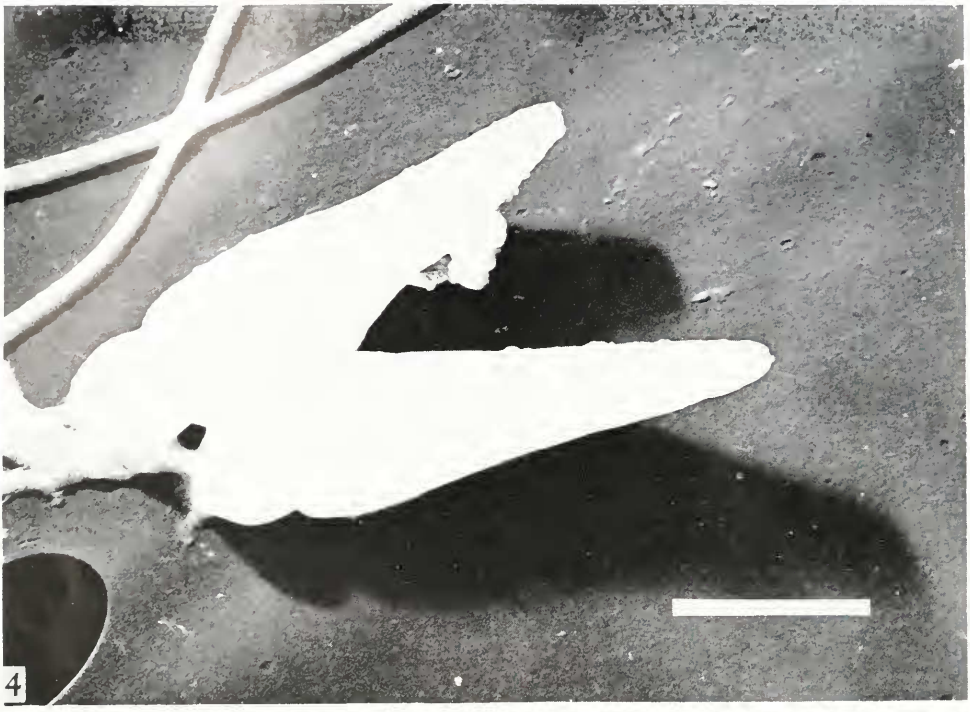
FIGURE 1. Electron micrograph of *Hemicentrotus pulcherrimus* spermatozoa, suspended for one minute in sea water and fixed with neutralized formalin.

FIGURE 2. *H. pulcherrimus* spermatozoa suspended in Ca-free artificial sea water for one minute, fixed with neutralized formalin.

FIGURE 3. Spermatozoa of *H. pulcherrimus* in Ca-free sea water, to which was added an equal amount of Ca-low egg-water. Fixed 30 seconds after the addition of egg-water, at the height of the agglutination reaction.





FIGS. 4-5.

lashing vigorously, and then they depart as suddenly as they arrived. In these small aggregates, as in the larger ones, the number of spermatozoa at any given time does not change materially. This behavior continues in the sample under the cover glass as long as the spermatozoa remain active, without respect to the reversal of agglutination in the main part of the suspension.

*Acrosome reaction in Ca-low sea water*

The easily observable fact that spermatozoa in a Ca-low medium do not adhere by their tips to the glass surfaces, even in the presence of egg-water, indicates that the acrosome reaction does not take place in such a medium. Phase contrast observation of individual acrosomes shows them all to be identical in appearance with those of freshly shed spermatozoa in pure sea water, and electron micrographs confirm this observation (Figs. 3, 10 and 12; compare with Figs. 1 and 8).

In order to differentiate between spermatozoa which had been involved in the clumps and those which had not, a strongly agglutinated suspension was hand-centrifuged to separate the clumped sperm from the freely swimming fraction. The sedimented clumps were re-suspended in fresh Ca-free sea water and examined for acrosome reaction. Both phase contrast and electron microscopic observation showed no difference between the acrosomes of these and of spermatozoa in the stock suspension (in Ca-low sea water before the addition of egg-water).

To control these observations, 0.36 *M* $CaCl_2$ was added to the Ca-low egg-water, making its calcium content equal to that of normal sea water (4%). This was then mixed with spermatozoa freshly suspended in sea water. Figures 4, 5, 6 and 7 show electron micrographs of spermatozoa of *H. pulcherrimus* fixed in pure sea water, immediately after the addition of egg-water, at the height of the agglutination reaction and after its reversal, respectively. Figures 8 and 9 show spermatozoa of *P. depressus* in sea water, and 30 seconds after the addition of egg-water.

*Fertilizing capacity after agglutination in Ca-low medium*

The fact has been repeatedly pointed out (Lillie, 1913; Tyler, 1941) that interaction with an agglutinating agent lowers the fertilizing capacity of a sperm suspension. The amount of diminution has been found to vary considerably from species to species and even among individuals within a given species, but in the writer's experience, also, there is always some reduction in fertilizing capacity following agglutination, when this takes place in a calcium-containing medium. It is equally clear, in the three sea urchin species under consideration, that there is no loss whatsoever in fertilizing capacity when the spermatozoa are agglutinated in Ca-low sea water. In this case, also, the clumped spermatozoa were quickly centrifuged out and re-suspended in fresh calcium-free medium, but no reduction in fertilizing capacity could be found after reversal of agglutination, either in these or in the

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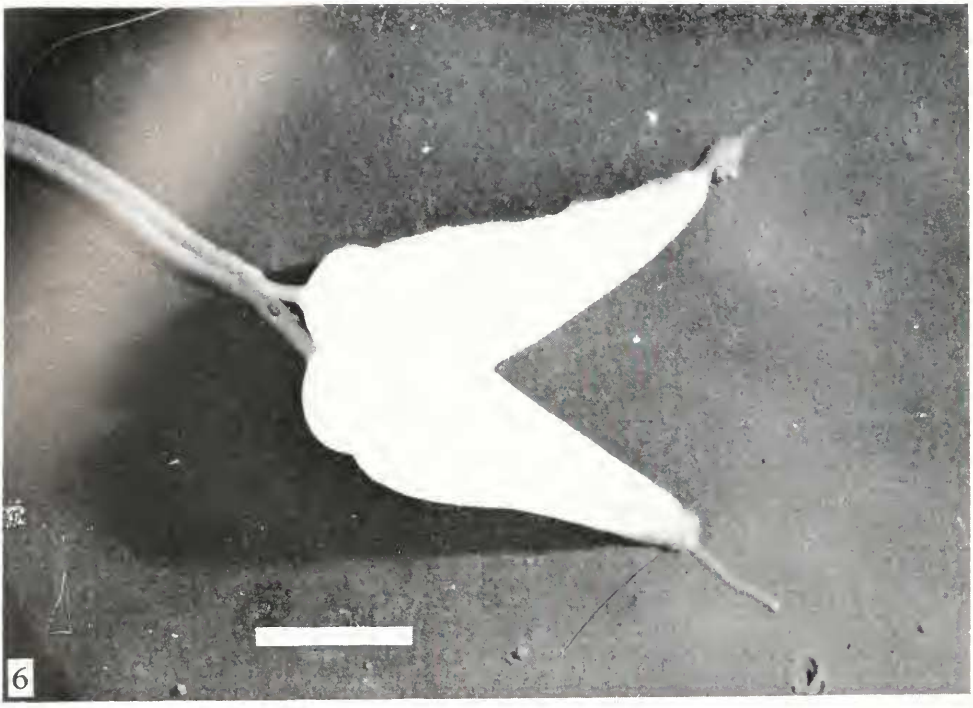
FIGURE 4. Spermatozoa of *H. pulcherrimus* in sea water, fixed with neutralized formalin. Chrome-shadowed electron micrograph.

FIGURE 5. *H. pulcherrimus.* Spermatozoa fixed two seconds after addition of homologous egg-water to sea water suspension. Breakage of acrosome filament commonly found in preparations fixed immediately after addition of egg-water (see Dan, 1952, Figs. 4 and 5).





FIGS. 6–7.

free-swimming fraction of the original suspension, when they were used to inseminate eggs in sea water.

In corresponding experiments with *H. pulcherrimus* spermatozoa agglutinated in sea water, it was found that while the supernatant fraction gave between 35 and 70% fertilization, similar dilutions of the clumped-reversed sperm gave between 0 and 3%.

## Discussion

These results include a number of aspects for which no explanation is forthcoming at present. On the other hand, there are certain points which stand out with unexpected clarity. The most striking of these is the failure of a typical acrosome reaction when sea urchin spermatozoa are reversibly agglutinated by homologous egg-water in the virtual absence of calcium ions. This complete separation of the two reactions, which are normally elicited simultaneously by the one stimulus of egg-water, calls for a reconsideration of the problem in more detail.

An acrosome reaction to the stimulus of egg-water, which he thought provided an explanation for the reversible agglutination phenomenon, was first reported by Popa in 1927. Using Janus green-stained Arabacia sperm, Popa found that a sticky substance was produced at the tips of the sperm heads. He believed that this substance held the sperm together in clusters, and that its dispersion accounted for the reversal of agglutination. Improved optical apparatus has shown, however, that the substance (or structure) extruded from the sperm acrosome has apparently the same shape and adhesive properties long after the agglutination clusters have broken up (Dan, 1952). Popa's idea thus loses a good deal of its plausibility, since it fails to explain reversibility.

There are, however, more fundamental faults to be found in this scheme, since close observation of reversibly agglutinated spermatozoa clearly indicates, as Jacques Loeb (1914) reported forty years ago (p. 127), " . . . that the spermatozoa at the periphery of a cluster are in free progressive motion. When the clusters were small or when the sperm suspension was thin it was possible to observe the spermatozoa which are in the center of the cluster. It was seen that the spermatozoa in the center also were in very lively motion, with the possible exception of small lumps or groups of spermatozoa which may have stuck together. The clusters reminded the writer of a dense swarm of insects which move like a coherent mass through space. These clusters move like one solid body through the water, notwithstanding the fact that the individual spermatozoa are free to scatter."

This easily observable freedom of movement on the part of the agglutinated spermatozoa, as well as the fact that the regular clusters begin to be formed the moment egg-water is introduced into a sperm suspension and attain a large size within a few seconds by the fusion of smaller clumps, almost certainly rules out any possibility that the acrosome reaction can provide a physical basis for re-

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Figure 6. *H. pulcherrimus* spermatozoa fixed 20 seconds after acrosome reaction. The substance which was extruded has dissipated, exposing the acrosome filament.

Figure 7. *H. pulcherrimus* spermatozoan fixed three minutes after addition of egg-water, 30 seconds after reversal of agglutination. The acrosome filament has the same appearance as in the previous sample, fixed at the height of agglutination.




FIGS. 8–10.

versible agglutination. There is obviously some other force which instantaneously orients the swimming spermatozoa into groups and holds them there for a predetermined period, at the end of which they again become free to resume their independent behavior. Whether this is a tropistic reaction, as Loeb rather doubtfully suggested, a chemical reaction between fertilizin molecules and antifertilizin on the sperm head, as Tyler proposes, or some entirely different sort of effect, the writer does not know.

This negative conclusion, that the acrosome reaction cannot provide a causal explanation of the regular, oriented clustering found in reversible agglutination, is emphasized by comparison with the process of irreversible agglutination which follows exposure to hyperalkaline sea water, some foreign egg-waters, and various serum- or other protein-containing solutions. In these media the entire spermatozoan becomes sticky, and the cells adhere to each other at random, the whole suspension gradually becoming immobilized. As Loeb has described it (1914): " . . . the spermatozoa at first stick together to form short rows or threads; and later the threads begin to stick together to form irregular networks. At no time is there any appearance of cluster formation."

It is quite conceivable that small, regular clusters might be formed if each acrosome should become only sticky enough to adhere to another sticky acrosome,[3] but the time required for such cluster formation would greatly exceed the one or two seconds within which cluster formation is achieved in these species, and such an explanation does not provide at all for the addition of the second and succeeding layers of spermatozoa, or for the fusion of small clusters to form large ones.

A second kind of evidence dissociating agglutination and the acrosome reaction is found in the fact that sea urchin acrosomes react to the non-specific stimuli of contact with solid surfaces and hyperalkalinity, in neither of which reversible agglutination is involved.

In the third place, there is the observation presented in this paper, that the minimum calcium requirement for reversible agglutination is very much lower than that for the acrosome reaction, so that it is possible to secure a typical agglutination reaction without any detectable response of the acrosomes.

Summarizing this evidence against a causal relation between the two reactions, we find that:

1. reversibly agglutinated spermatozoa are freely moving within the clusters, and therefore cannot be considered to be held together by any substance released from the acrosome;
2. there is no significant difference, in either living or fixed sperm, between the appearance of the acrosomes at the height of agglutination and after reversal;

[3] This may be the explanation of "rosettes" which are formed by the sperm of some echinoderms (*e.g., Mespilia globulus*) at the air-water and water-glass interfaces as a response to homologous egg-water.

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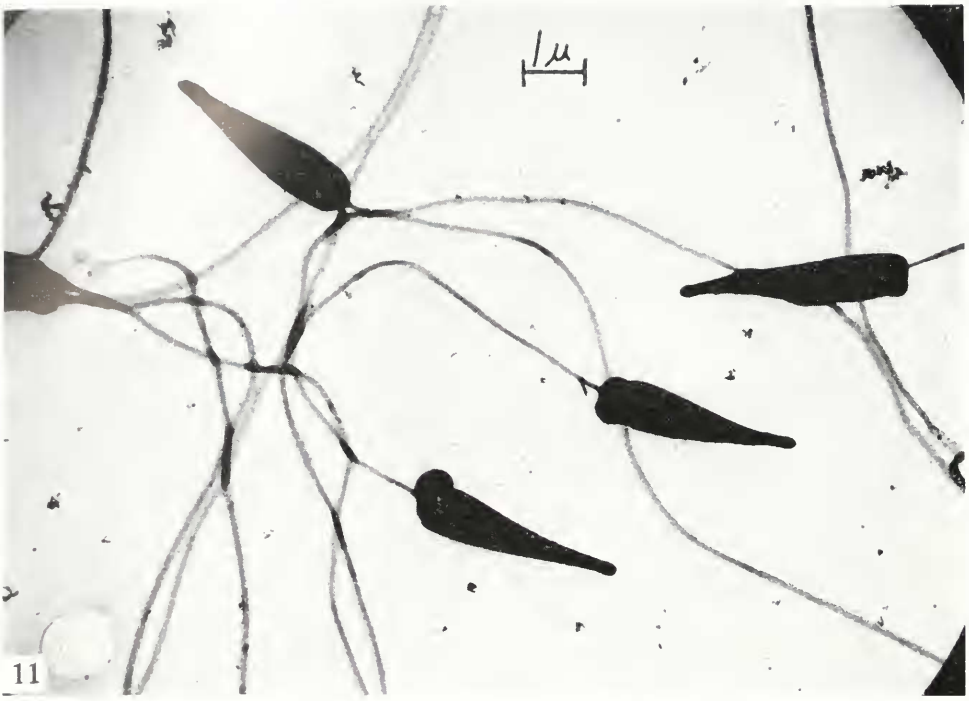
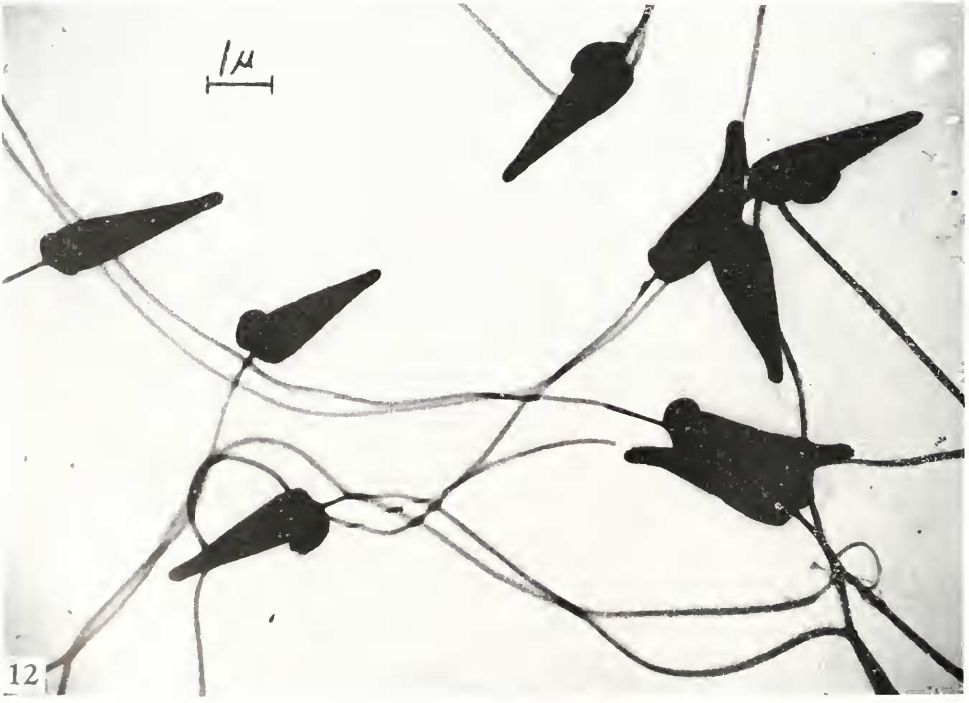
FIGURE 8. Spermatozoa of *Pseudocentrotus depressus,* fixed after one minute in sea water.
FIGURE 9. Acrosome reaction of *P. depressus,* in response to homologous egg-water.
FIGURE 10. Spermatozoa of *P. depressus* suspended in sea water, and fixed 30 seconds after addition of Ca-low egg-water. Note lateral shift of middle pieces; this condition is also usually found in Ca-free suspensions before addition of egg-water.




FIGS. 11–12.

3. the clusters appear almost instantaneously after the addition of egg-water, which seems to preclude the possibility that they are formed by random acrosome-to-acrosome collisions. Moreover, this explanation would include only the smallest clusters;
4. the acrosomes react to stimuli which do not cause reversible agglutination, such as contact with solid objects and hyperalkalinity;
5. in the virtual absence of calcium, the stimulus of egg-water causes agglutination without any acrosome reaction.

Another, and perhaps more important, fact which emerges from the results of these experiments is that reversible agglutination without acrosome reaction has no effect on the fertilizing capacity of the spermatozoa. Lillie (1913) first showed that sperm suspensions which had been agglutinated by fertilizin had undergone a considerable (ca. 50%) reduction in fertilizing capacity. Tyler (1941), repeating Lillie's work, found a much greater loss of fertilizing capacity (p. 196): "Insemination with amounts of sperm that were well above the control minimum for 100 per cent fertilization gave in all tests with the agglutinated and reversed sperm between 0 and 3 per cent fertilization." [4] Rothschild (1947) has suggested that reversible agglutination may be (p. 241) "a form of false fertilization between spermatozoa instead of between eggs and spermatozoa; or at any rate, that there is some reaction between the surfaces of the spermatozoa which is sufficiently similar to that which occurs between an egg and a spermatozoon to alter the spermatozoon in an irreversible manner." Spermatozoa which have thus lost their fertilizing capacity following agglutination, Rothschild has characterized as being "muzzled" as the result of exposure to fertilizin in solution.

Among the three Misaki sea urchin species which undergo reversible agglutination, the writer has found considerable variation in the fertilizing capacity of reversed spermatozoa, especially if they are used for insemination immediately after reversal of agglutination. However, in every case there is a significant reduction in fertilizing capacity after agglutination in normal sea water, in which a majority of the acrosomes have reacted, and no reduction at all when agglutination has taken place in a calcium-low medium, where none of the acrosomes has reacted.

This result strongly suggests a causal linkage between the acrosome reaction and the fertilization reaction. Within a strict time limit, it appears that suspensions of spermatozoa with intact acrosomes possess full fertilizing capacity, while in those in which the acrosomes have reacted to such a stimulus as egg-water, this capacity is reduced. The suggestion fairly makes itself, that this acrosome reaction

[4] In these experiments, Tyler used very strong egg water, such that "agglutination usually lasted 30–40 minutes" (loc. cit.). Loeb (1914) gives 2–10 minutes as the range of duration of the agglutination reaction in the species used by Tyler (*Strongylocentrotus purpuratus*); the Misaki species also fall within this range. The possibility should be considered that egg-water strong enough to cause such extreme agglutination may have had a toxic effect on the spermatozoa.

FIGURE 11. Spermatozoa of *P. depressus* fixed 10 minutes after suspension in Ca-free sea water.

FIGURE 12. Spermatozoa of *Anthocidaris crassispina* suspended in Ca-free sea water and fixed one minute after addition of Ca-low egg-water, at height of agglutination reaction.

in response to fertilizin is the "false fertilization" of Rothschild, and its completion at a distance from the egg surface constitutes the muzzling effect on the spermatozoa.

The studies of Yamamoto (on fish eggs) and of Moser, cited above, have shown that the series of linked reactions constituting activation, the response of the egg, breaks down when calcium is excluded from the medium. The observations presented in this paper can be taken to indicate, however, that the stimulus itself is lacking, because of the failure of the acrosome reaction, when insemination takes place in a calcium-free medium.

Reference was made earlier to the change in position of the middle piece, which often occurs in all spermatozoa after a short exposure to plain Ca-low medium, without causing any diminution in the fertilizing capacity of the suspension. This same effect has been reported by Tyler (1952), who observed it in spermatozoa, suspended in sea water, which had been exposed to homologous egg-water. Tyler interprets this as an evidence of a general loosening of the sperm structure, preparatory to the complete separation of its parts within the egg cytoplasm. In this connection may be mentioned a similar relaxation of tension which occurs in starfish spermatozoa at the time of the acrosome reaction (Dan, 1954).

It is, moreover, almost certainly this same shifting of the middle piece under the influence of egg-water and various vital stains which was observed by Popa (1927) in *Arbacia* spermatozoa, although he described it as the formation of a "lateral body." However, his drawings show the middle piece tending to move around the basal end of the sperm head, and, significantly, his final figure representing the extruded "lateral body" at the side of the nucleus, shows a sperm head without any (other) middle piece.

## Acknowledgment

It is a pleasure to acknowledge again the willing cooperation of the staff of the Misaki Marine Biological Station, and the kindness of Prof. A. Takamiya, of Tokyo Institute of Technology, who arranged for the use of the Institute's electron microscope. The photography was done by Mr. H. Akabori.

## Summary

1. When sea urchin spermatozoa are suspended in Ca-free artificial sea water,

a) they show no morphological change, except that there is a tendency for the middle piece to become "loose" and move around to the side of the nucleus;
b) the sperm swim vigorously and attack the surface of unfertilized eggs as in normal sea water, but without being able to penetrate;
c) they do not undergo the acrosome reaction on contact with glass or collodion surfaces.

2. If "Ca-free" egg-water is added to such a sperm suspension,

a) a regular, reversible agglutination reaction occurs, which, with strong egg-water, lasts 2–4 times longer than in a corresponding suspension in which the calcium content is adjusted to that of normal sea water;

b) no acrosome reaction can be detected in spermatozoa thus agglutinated in the virtual absence of calcium;

c) after reversal of agglutination, such spermatozoa show no reduction in fertilizing capacity when used to inseminate unfertilized eggs in sea water. This is in marked contrast to the sea water control, in which there is a significant loss in fertilizing capacity following agglutination.

3. It is concluded that:

a) the agglutination reaction and the acrosome reaction must be considered to be separate phenomena, both occurring in response to the stimulus of egg-water, but not causally interrelated;

b) in view of the fact that fertilizing capacity is retained to the full extent by sperm suspensions in which the acrosomes remain intact, and is at least partially lost following mass acrosome reaction, this acrosome reaction must play an important role in the process of fertilization.

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